silk solution probably as a result of their ability to encourage the aggregation of the fibroin chains by binding to them and this is thought to be disadvantageous in the formation of strong and stiff fibroin gels.

[0181] It is considered that it may be of further advantage to use cocoon or raw silks degummed with trypsin in ammonium carbonate buffer at 40° C.

[0182] Gelling

[0183] The optimised regenerated fibroin solution was gelled by exposure to an acetic acid solution, or an acidic buffer or to acetic acid vapour. The concentration of the acidic buffer and length of exposure to it were crucial to the pore size and the strength and stiffness of the resulting gel. Too concentrated acetic acid solutions or prolonged exposure to acidic buffers resulted in over-gelation of the fibroin.

[0184] Freezing under-gelled fibroin resulted in a reduction in the pore size and weaker scaffolds while strong over-gelation gave non-porous gels containing a low density of large splits produced by large ice crystals. It was found that the length of exposure and concentration of the acidic buffer or vapour required for optimal gelation depended on the geometry and size of the fibroin cast. Thus longer treatments were required to optimally gel fibroin in moulds constructed from 20 mm diameter dialysis tubing compared with 10 mm dialysis tubes.

[0185] It was found to be advantageous to gel 8-10% w/v optimised regenerated silk fibroin solution prepared from trypsin degummed silk contained in 10 mm diameter dialysis tubes for 2.5 hours to 5 hours with aqueous 1% w/v acetic acid at 20° C. In contrast similar solutions prepared from alcalase degummed silk required gellation for 16 hours under the same conditions. The greatly extended treatment time for alcalase, compared with trypsin degummed silk probably results from much greater degradation of the fibroin chains in the former (see above).

[0186] It will be understood that several agents can be used to facilitate the formation of a hydrogel from the improved regenerated silk solution. By way of example only these include acetic acid solutions, acetic acid vapour, other acidic solutions or buffers or vapours, solutions containing calcium ions, surfactants, heating, ultraviolet light, laser radiation, microwave radiation, ultrasound, low frequency vibrations, dilution of the fibroin beneath 5% w/v, concentration of the fibroin beyond 10% w/v, mechanical strain, shear forces, and extensional flow. These agents can be used singly or in combination of two or more agents.

[0187] It will be understood that compared with regenerated fibroin solutions prepared by the standard protocol disclosed in the literature, the reduced degradation of the optimised regenerated fibroin solution, its greatly shortened gelation time and its heightened sensitivity to extensional flow and shear make it highly advantageous for extrusion into strong filaments.

[0188] Compared with that prepared by the standard protocol, the optimised regenerated fibroin solution is also highly advantageous for coatings, for forming beads and microspheres, for encapsulation, as an adhesive, for casting of films, and for incorporation into composite materials.

[0189] Freezing

[0190] For the preparation of porous implantable material the optimised regenerated fibroin solution after gelling can be rendered porous by freezing. Freezing is thought to result in phase separation of a fibroin-rich phase from a fibroin-poor phase and ice crystal formation in the latter. These two mechanisms are thought to combine to give rise to a high density of interconnected pores in the gels.

[0191] The branching dendritic pattern of ice crystals formed in this way is reflected in the orientation of the approximately ellipsoidal pores and the distribution of the interconnections between the pores. The walls of these pores are strongly birefringent. The sign of the birefringence shows that the fibroin molecular chains are highly aligned circumferentially around the pore walls. This suggests that freezing strains and orientates the molecules in the pore walls. It will be understood that the orientation of the fibroin obtained in this way contributes beneficially to the mechanical properties of the material.

[0192] The freezing step also makes the fibroin in the pore walls insoluble in water and most other aqueous solvents suggesting that it has been partially converted to the insoluble silk II state in which intra- and inter-molecularly bonded beta-sheets predominate. This transition to the silk II state may result from the removal of water from the protein chains produced by a combination of phase separation and their alignment and pulling together, both as a consequence of ice crystal formation. Thus the formation of the insoluble silk II state rather closely mimics the natural process by which silks are extruded which also depends on phase separation, loss of water from the fibroin-rich phase and strain dependent orientation and silk II formation.

[0193] For a single freezing cycle, the temperature of the freezing step has a small effect on the pore size with the largest pores produced by freezing between −12° C. to −16° C. Varying the temperature and including low concentrations of antifreezes or sugars in the regenerated protein solution can be used to vary the ice crystal size and morphology and hence the size and shape of the pores in the material.

[0194] Increasing the number of freezing cycles produced an increase in the size of the pores as a result of damage by ice crystals. This was accompanied by some loss in the stiffness and strength of the final material.

[0195] Zone freezing gives advantageous control over the shape and orientation of the ice crystals and hence the shape and orientation of the pores. Defining the points, loci or planes at which zone freezing is initiated provides a means of controlling the branching pattern of ice crystal formation and hence the pattern of orientation of the ellipsoidal pores and the distribution of the interpore connections in the fibroin material. It will be understood that this enables porous fibroin scaffolds to be produced in which the arrangement of pore walls mimics the arrangement of extracellular materials in tissues.

[0196] Thus, for example, zone freezing a thin slab of fibroin hydrogel placed on a single cold plate lying in the plane of the future osteochondral surface gives an anisotropic branching and radiating pattern to some extent resembling the arrangement of collagen fibres in the deep layers of cartilage. It is to be understood that scaffolds mimicking the tissue plan of other tissues could be made in this way.

[0197] It will be understood that methods other than gelation and freezing can be used to introduce intercommunicating pores into the optimised regenerated fibroin solution. By way of example only these include salt leaching and gas foaming.

[0198] Treatment with Ethanol Solution

[0199] Treating the porous fibroin hydrogel with an aqueous ethanol solution after freezing, is thought to facilitate the formation of beta sheet inter and intramolecular hydrogen